Effect of water stress and diclofop-methyl on photosynthesis, carotenoid and abscisic acid content of leaves of *Avena byzantina* and *Avena fatua*

A.K. Cowan*, S.L. Turner and C.E.J. Botha

Department of Botany, Schönland Botanical Laboratories, Rhodes University, Grahamstown 6140, Republic of South Africa

Received 23 September 1994; revised 21 October 1994

The effect of combined water stress and diclofop-methyl treatment on photosynthesis and carotenoid and abscisic acid (ABA) content of leaves of *A. byzantina* and *A. fatua* was investigated. Sublethal doses of diclofop-methyl caused a transient decline in net assimilation rate and a decrease in β-carotene and zeaxanthin in leaves of both species. The decline in carotenoid levels occurred concomitant with a substantial but transient increase in ABA. A similar but less dramatic trend was observed for water-stressed plants. Recovery of photosynthesis in seedlings exposed simultaneously to diclofop-methyl and water stress, was associated with an increase in β-carotene and zeaxanthin content and a return to basal ABA levels in leaves of *A. byzantina*. By comparison, substantial accumulation of zeaxanthin was observed in leaves of *A. fatua* following combined water stress and herbicide treatment, apparently at the expense of ABA. Similar findings were made regarding levels of zeaxanthin when diclofop-methyl was applied to already water-stressed plants of *A. byzantina* and *A. fatua*. It is proposed that herbicide- and/or water-stress-induced alterations in acetyl-coenzyme A carboxylase activity coupled with reduced demand for fatty acid synthesis, facilitate channelling of photosynthetically fixed carbon into isoprenoids and that alterations in the capacity for terpenoid synthesis forms part of the mechanism by which drought stress antagonizes the activity of aryloxyphenoxypropionic acid herbicides.

Die invoed van gekombineerde waterstremming en diklofopmetiel-behandeling op die karotenoïed- en ABA-konsentrasie van blare van *A. byzantina* en *A. fatua* is ondersoek. Subletale dosisse van diklofopmetiel het tot 'n tydelike verlaging in die netto assimilasietempo en tot 'n verlaging in β-karoteen en zeaxantien in blare van albei spesies gelei. Die afname in karotenoïedvlakke het plaasgevind saam met 'n sterk maar tydelike verhoging in ABA. 'n Soortgelyke maar minder opvallende neiging is waargeneem in plante wat aan waterstremming onderhewig was. In saailinge van *A. byzantina* wat aan beide diklofopmetiel en waterstremming blootgestel is, het die herstel in fotosintese plaasgevind saam met 'n verhoging in β-karoteen en zeaxantienvlakke en 'n herstel van die ABA na die basale vlak. In die geval van *A. fatua* wat aan diklofopmetiel en waterstremming blootgestel is, is 'n aansienlike verhoging in zeaxantien waargeneem, skynbaar ten koste van ABA. Soortgelyke waarnemings is gemaak met betrekking tot zeaxantienvlakke nadat diklofopmetiel toegedien is aan reeds watergestremde plante van *A. byzantina* en *A. fatua*. Daar word voorgestel dat herbisied- en/of waterstremming-geïnduseerde veranderings aan ACCase-aktiwiteit, gekoppel aan 'n verlaagde aanvraag vir vetsuursintese, tot die omskakeling van fotosintese-gefikseerde koolstof na isoprenoïede lei. Wysigings in die vermoë om isoprenoïede te vorm, kan 'n deel uitmaak van die meganisme waarvolgens droogtestremming die aktiwiteit van AOPP herbisiede beïnvloed.

Keywords: Abscisic acid; *Avena*; carotenoids; diclofop-methyl; herbicide; water-stress; wild oat.

Abbreviations: ABA, abscisic acid; ABA-GE, abscisic acid glucose ester; ACCase, acetyl-coenzyme A carboxylase; AOPP, aryloxyphenoxypropionic acid; diclofop-methyl, methyl-(±)-2-[4-(2,4-dichlorophenoxy)-phenoxy]-propanoic acid; DPA, dihydrophaseic acid; GC-EC, gas chromatography-electron capture; HPLC, high-performance liquid chromatography; IRGA, infrared gas analyser; MCCase, methyl crotonyl-coenzyme A carboxylase; NAR, net assimilation rate; PA, phaseic acid; PCCase, propionyl-coenzyme A carboxylase; RWC, relative water content; TLC, thin-layer chromatography.

* To whom correspondence should be addressed at: Department of Horticultural Science, University of Natal, Pietermaritzburg, Scottsville 3209, Republic of South Africa

Introduction

Wild oat (*Avena byzantina* K. Koch and *A. fatua* L.) are widespread and economically important weeds in winter wheat-growing regions of southern Africa. Currently, AOPP graminicides such as diclofop-methyl and fenoxaprop are used in the chemical control of wild oat. Field observations, however, indicate that the activity of these post-emergence herbicides is markedly reduced following exposure of *A. fatua* to periods of water stress (Field & Caseley 1987; Dastgheib *et al.* 1990; Rossi *et al.* 1993). Possible reasons for a reduction in herbicide efficacy in *A. fatua* under water stress include a decline in herbicide uptake (Akey & Morrison 1983), a decrease in extention growth (Andrews *et al.* 1989), and subsequent reduction in demand for membrane synthesis (Dastgheib *et al.* 1990) and alterations in metabolism of the applied herbicide (Rossi *et al.* 1993).

AOPP herbicides and in particular diclofop-methyl, specifically inhibit the biotin-containing plastid-localized enzyme ACCase (EC 6.4.1.2) which catalyses the ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA in fatty acid biosynthesis (Focke *et al.* 1991; Kobek *et al.* 1988; Lichtenthaler 1990). The consequence of inhibition in susceptible plants is lack of glycerolipid and biomembrane formation in meristematic tissue which ultimately causes cell death. The cascade of events leading to death in treated plants was recently outlined by Cobb (1992). These are, firstly, accumulation of the toxic form of the graminicide in meristematic tissue; secondly, interference with lipid synthesis and plastid development; thirdly, cessation of growth and function of meristems and the development of chlorosis. Finally, death of the treated plant occurs approximately

three weeks after application of the graminicide.

Inhibition and/or reduced activity of ACCase would be expected to increase the acetyl-CoA pool available for plastid isoprenoid synthesis. AOPPs also block PCCase but not MCCase (EC 6.4.1.4), which may be involved in isoprenoid metabolism (Motel *et al.* 1993). The latter enzyme has been implicated in the metabolism of leucine and as a component of the 'mevalonate shunt' (Popjak 1971). MCCase has recently been purified to homogeneity from several higher plant tissues (Alban *et al.* 1993; Chen *et al.* 1993; Diez *et al.* 1994). It is, therefore, not unreasonable to assume that isoprenoid synthesis would continue in treated plants, at least during the early stages following herbicide application. Isoprenoids, and in particular sterols and carotenoids, have been implicated in the protection of plant membranes. For example cholesterol, which is a ubiquitous constituent of eucaryote phospholipid membranes, maintains lipid order and condenses and reinforces the bilayer (Ourisson 1994). Plant carotenoids, in addition to their well-characterized role in photosynthesis, are purported to serve a similar function (Subczynski *et al.* 1993).

It is now well established that plants exposed to a water deficit accumulate the terpenoid phytohormone, ABA (Zeevaart & Creelman 1988). Most of the available evidence suggests that under these conditions the accumulated ABA is derived from a carotenoid precursor. In this regard, perhaps the most significant observations include the demonstration of the origin of ABA from zeaxanthin (Rock & Zeevaart 1991; Duckham *et al.* 1991) and all-*trans*-β-carotene (Cowan & Richardson 1993). Zeaxanthin, a component of the xanthophyll cycle, plays a major role in dissipating excess energy under conditions where absorption of light exceeds the capacity of the chloroplast to utilize the products of photochemical reactions (Demmig-Adams & Adams 1992). Furthermore, zeaxanthin has been observed to increase order and decrease motional freedom of lipid alkyl chains in fluid-phase membranes (Subczynski *et al.* 1993). The proposed biosynthetic interrelationship between zeaxanthin and ABA suggests that these components act in concert to facilitate acclimation. Changes in ABA and/or genetic differences in the ability to produce ABA play an important role in determining plant vigour and hence increased capacity to tolerate abiotic and biotic pressures (Quarrie 1991).

At present, little is known of the effect of diclofop-methyl on the carotenoid and ABA content of treated plants. In water-stressed plants the increase in ABA is reported to be associated with a concomitant decline in xanthophylls in an apparent 1:1 ratio on a molar basis. Whether this is a characteristic of all plants exposed to a water stress, and in particular monocotyledonous species, remains to be determined. Nevertheless, the present study examines the effect of diclofop-methyl and water stress on changes in carotenoid and ABA levels in an attempt to determine whether stress-induced alterations in terpenoid metabolism contribute to the reduced susceptibility of wild oat to diclofop-methyl.

Materials and Methods

Plant material

Seeds of *A. fatua* L. and *A. byzantina* K. Koch were kindly supplied by Hoechst South Africa (Pty) Ltd., Randburg. Dormancy was broken by imbibition in a solution of GA_3 (1 g/l) for 1 h at room temperature and the seeds were then immediately planted 1.5 cm deep in 12.5 cm (diameter) pots (3 seeds/pot) containing potting soil:vermiculite, 4:1 (v/v). Plants were grown in a phytotron under natural irradiance supplemented with metal halide lamps to provide a 14-h photoperiod, and a 20/15°C light/dark cycle at 60% relative humidity. Pots were subirrigated daily with water and once a week with 30% Hoagland's nutrient solution.

Herbicide treatment

Unless otherwise stated, uniform 28-day-old seedlings in the three- to five-leaf stage of development were selected for treatment. The commercially formulated graminicide HOELON (containing 284 g/l diclofop-methyl) obtained from Hoechst South Africa (Pty) Ltd., was diluted with distilled water to 80 μM and a 10 μl aliquot (equivalent to 272 ng) was applied directly to the axial region of the second leaf of each plant using a micropipette. Plants were maintained in the phytotron until analysis.

Water stress

Water stress was imposed by withholding water from 28-day-old seedlings of uniform developmental stage. Plant water status was measured by determining leaf RWC, after consideration of associated problems as discussed by Bennett (1990). Data are expressed as % RWC = [(fresh wt. – dry wt.)/(turgid wt. – dry wt.)] × 100.

Combined diclofop-methyl and water stress effects

In order to determine the combined effects of water stress and herbicide treatment on photosynthetic rate, carotenoid and ABA content of wild oat, two approaches were adopted. The first involved simultaneous application of the herbicide and imposition of water stress, while the second involved imposing a water stress until % RWC of leaves had been reduced by approximately 15% (about 14 days) before application of the herbicide.

Gas exchange analysis

Net assimilation rates were obtained from analysis of leaf gas exchange. Plants were transferred from the phytotron and placed in the laboratory under conditions of constant illumination (800 $\mu mol.m^{-2}.s^{-1}$) and temperature (20 ± 0.5°C) at 350 p.p.m. CO_2. Following an equilibration period of 1 h, CO_2 measurements were taken using an ADC LCA-2 portable infrared gas analyser (The Analytical Development Co. Ltd, Hoddesdon, U.K.) at 10-min intervals from the second, third and fourth leaf of each plant (3 plants/pot). The IRGA was equipped with an ADC DL-2 datalogger coupled to an ADC ASUM 2 mass flow meter. A Parkinson narrow-leaf chamber, coupled to a refrigeration unit, was connected to the IRGA. Actinic light was provided by a 400-W high-pressure sodium lamp placed 25 cm above the leaf chamber. Atmospheric air, pumped into a buffer drum from outside the laboratory, was fed into the analyser by the mass flow meter at 350 ml/min in an open circuit.

Light response curves were constructed for both *A. fatua* and *A. byzantina* from gas exchange data obtained from constant-temperature experiments in which light intensity was varied using shade-cloth placed between the leaf chamber and the light source. Light intensity, measured as photosynthetic photon flux density (PPFD), was decreased at 10-min intervals from 1500 to 0 $\mu mol.m^{-2}.s^{-1}$. Plots were constructed using the monomolecular equation described by Causton and Dale (1990). Light saturation points thus obtained were used in all subsequent experiments.

Carotenoid analysis

Leaf material from treated and non-treated plants was routinely harvested at mid-photoperiod to ensure maximum levels of zeaxanthin. Tissue was immediately frozen in liquid nitrogen, powdered and extracted with ice-cold methanol/ethyl acetate (50:50, v/v) containing butylated hydroxytoluene (20 mg/l) as an antioxidant. Extracts were filtered through 0.2 μm Spinex centrifugal filters, reduced to dryness under N_2 and resuspended in 2 ml of 70% methanol containing 0.08 g celite. Methanolic suspensions were applied to Sep-pak C_{18} cartridges and the carotenoids were eluted with 10 ml of 100% acetone. Individual carotenoids were separated by reversed-phase HPLC and zeaxanthin and β-carotene were identified and quantified as previously described (Afitlhile *et al.* 1993).

Analysis of ABA, PA, DPA and ABA-GE

Leaf material was extracted as described above and an aliquot of

high specific activity [^{3}H]-ABA (obtained from Amersham International, Buckinghamshire, U.K.), [^{3}H]-PA and [^{3}H]-DPA [prepared biosynthetically as decribed by Cowan and Railton (1987)] was added to correct for recovery. Extracts were filtered and reduced to dryness *in vacuo* at 30°C. The residue was resuspended in 0.5 M phosphate buffer (pH 7.5) and partitioned three times against equal volumes of diethyl ether. The aqueous fraction was then adjusted to pH 2.5 and further partitioned three times against equal volumes of ethyl acetate. Acidic ethyl acetate fractions were purified by TLC on silica gel GF_{254} in toluene:ethyl acetate:acetic acid (25:15:2, v/v). Zones corresponding to authentic ABA, PA and DPA were eluted from the gel and methylated with excess ethereal diazomethane. Methyl ester derivatives were subsequently purified by TLC in n-hexane:ethyl acetate (1:1, v/v) and quantified by GC-EC.

For analysis of conjugated ABA, aqueous fractions remaining after partitioning were treated with base at pH 11.0, incubated at 60°C for 1 h and then processed as described above.

Quantification by GC-EC was performed on a Perkin-Elmer 8310 instrument (EC source ^{63}Ni) using a wide-bore fused-silica column (15 m × 0.53 mm i.d.) of SPB-1 (Supelco, Bellefonte, PA) programmed from 160°C at 5°C per min with N_2 as carrier (linear gas velocity of 28 ml/min).

Statistical analysis

Two-way analysis of variance was carried out to test the degree of significance at the 95% level between stressed and non-stressed plants treated with diclofop-methyl. The null hypothesis (H_0) adopted was that results of all treatments were identical (H_0:$T_1 = T_2$), with the alternative hypothesis H_A:$T_1 \neq T_2$. Additionally, student *t*-tests were done on the corresponding data points from stressed and non-stressed treatments at each time interval.

Results

Gas exchange characteristics

The light response curves for *A. byzantina* and *A. fatua* are shown in Figure 1.

The light compensation point for both species was between 50 and 100 PPFD. *Avena byzantina* was only light-saturated at levels above 1500 PPFD (Figure 1A), whereas light saturation was achieved in the range 750–1000 PPFD for *A. fatua* (Figure 1B). Neither species showed light inhibition of net assimilation over the range of light intensities measured.

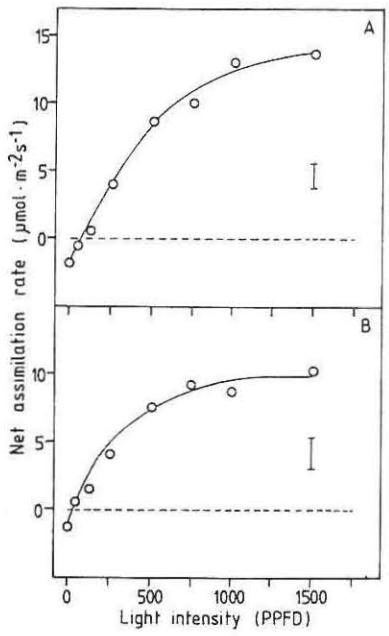

Figure 1 Light-response curves of *A. byzantina* (A) and *A. fatua* (B). Assimilation rates were determined at constant temperature and 350 p.p.m. CO_2. Light intensity was decreased from 1500 to 0 PPFD using shade cloth. Data are the mean of three independent experiments ± SE.

Carotenoid and ABA content

The results presented in Table 1 show the ABA, PA, DPA, ABA-GE, β-carotene and zeaxanthin content of leaves of *A. byzantina* and *A. fatua* cultivated under control conditions. Under these growing conditions, *A. fatua* contained significantly higher basal levels of β-carotene and zeaxanthin than *A. byzantina*, but lower levels of ABA and associated catabolic products. These two species therefore differed in their capacity to metabolize ABA.

Effects of simultaneous diclofop-methyl and water stress treatment

Following application of sublethal doses of diclofop-methyl to leaves of *A. byzantina*, a rapid decline in net assimilation rate was observed (Figure 2A). This decline in photosynthetic rate was, however, less marked in herbicide-treated plants exposed to a simultaneous water stress. A similar, although less dramatic effect was observed for *A. fatua* (Figure 2B), and the difference between stressed and non-stressed plants was also less accentuated. Interestingly, the recovery time (i.e. time taken to return to basal photosynthetic rate) was significantly shorter for plants of both species exposed simultaneously to herbicide and water stress.

Inhibition and recovery of photosynthesis correlated closely with changes in β-carotene in both species irrespective of treatment (Figure 2C & D). Plants exposed to both herbicide and water stress accumulated β,β-carotenoids following recovery of photosynthesis. In *A. byzantina*, both β-carotene and zeaxanthin levels increased under water stress (Figure 2C & E), whereas in water-stressed *A. fatua*, significant accumulation of zeaxanthin was observed (Figure 2D & F).

The leaf ABA content of control plants for both *A. byzantina* (Figure 3A) and *A. fatua* (Figure 3B) rose dramatically in response to diclofop-methyl treatment. Maximum levels were reached approximately 8 days after herbicide application. Thereafter ABA content declined, presumably due to catabolism, until basal levels were reached. A similar trend was observed for leaves of plants exposed to a simultaneous water stress. However, following an initial increase, ABA content was reduced to below pretreatment level. This response appeared more marked in *A. fatua* (Figure 3B).

Diclofop-methyl effects following water stress

When sublethal doses of diclofop-methyl were applied to leaves of *A. byzantina* and *A. fatua* plants already experiencing a water

Table 1 The carotenoid and ABA content of leaves (in nmol/g tissue) of non-stressed *A. byzantina* and *A. fatua*[a]

	β-carotene	Zeaxanthin	ABA	PA	DPA	ABA-GE
A. byzantina	733.41	66.67	8.89	2.71	0.91	4.64
	±31.87	±9.11	±0.54	±0.51	±0.07	±0.11
A. fatua	938.67	92.31	7.23	1.37	0.63	3.01
	±45.01	±12.03	±0.63	±0.13	±0.18	±0.17

[a] Compounds of interest were analysed as described in 'Materials and Methods'. Data represent the mean of three cultivations and extractions ± SE.

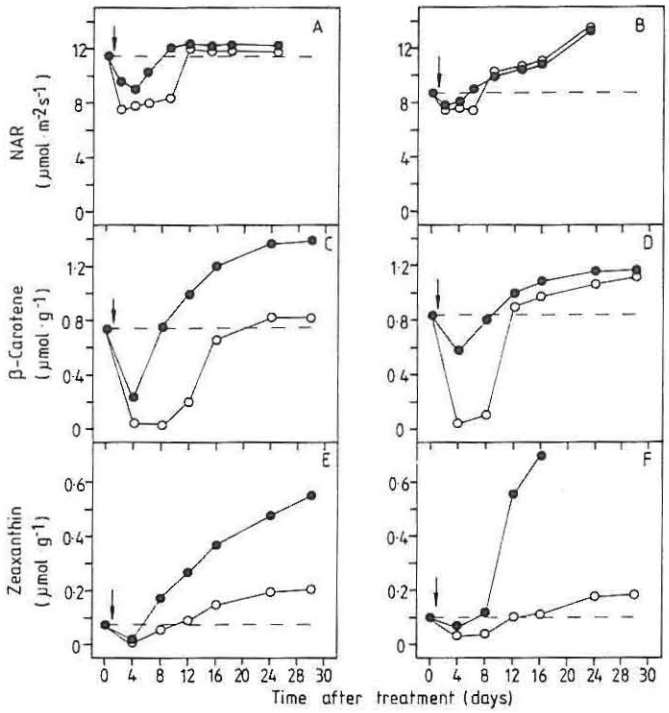

Figure 2 Changes in net assimilation rate and β-carotene and zeaxanthin content of leaves from stressed (closed symbols) and non-stressed (open symbols) *A. byzantina* (left panel) and *A. fatua* (right panel) following diclofop-methyl application. Arrows indicate the time at which diclofop-methyl was applied to seedlings. Water stress was imposed by withholding water from 28-day-old seedlings ($t = 0$) and leaves were analysed at the specified intervals. Data are representative of two trials each with three replicates per treatment. Analysis of variance of the data showed significant differences ($P < 0.05$ in all cases) between the treatments and a student t-test confirmed these differences at $t = 2$ to $t = 8$ for both species ($P < 0.05$).

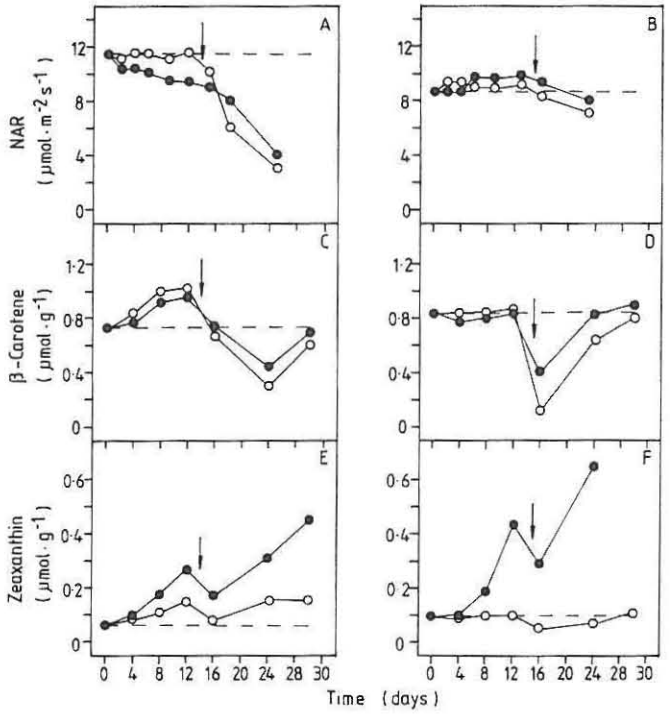

Figure 4 Effect of diclofop-methyl on net assimilation rate and β-carotene and zeaxanthin content of leaves from stressed (closed symbols) and non-stressed (open symbols) *A. byzantina* (left panel) and *A. fatua* (right panel). Arrows indicate the time at which diclofop-methyl was applied to seedlings. Water stress was imposed by withholding water from 28-day-old seedlings ($t = 0$) and leaves were analysed at the specified intervals. Data are representative of two trials each with three replicates per treatment. Analysis of variance of the data showed no significant differences between the treatments in A, B, C and D ($P > 0.05$ in all cases). Significant differences in zeaxanthin (E & F) were observed between stressed and non-stressed leaves ($P < 0.05$).

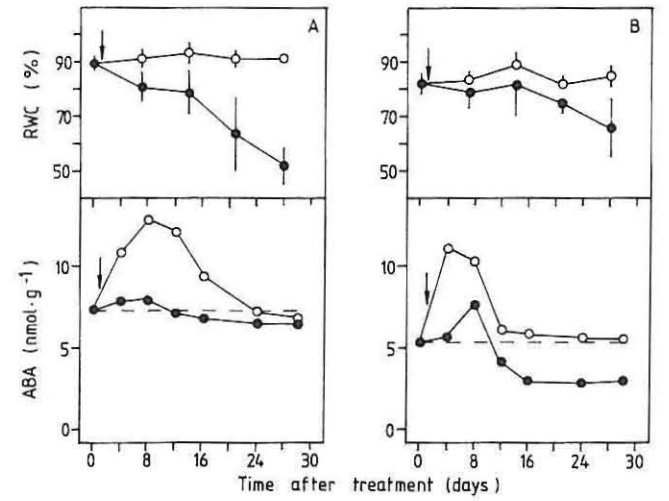

Figure 3 Changes in ABA levels and percentage RWC of leaves from stressed (closed symbols) and non-stressed (open symbols) *A. byzantina* (A) and *A. fatua* (B) following treatment with diclofop-methyl. Conditions were as described for Figure 2.

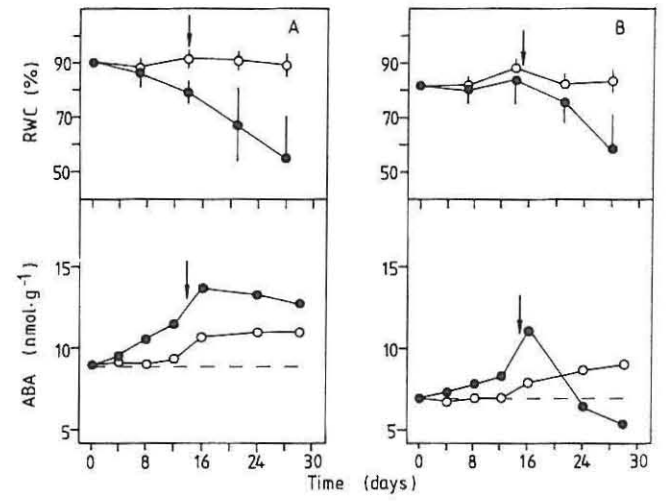

Figure 5 Changes in ABA levels and percentage RWC of leaves from stressed (closed symbols) and non-stressed (open symbols) *A. byzantina* (A) and *A. fatua* (B), subsequently treated with diclofop-methyl. Conditions were as described for Figure 4.

stress, a decline in photosynthetic rate (Figure 4A & B) and β-carotene content (Figure 4C & D) was observed. The zeaxanthin content of both species increased in response to herbicide treatment under water stress conditions (Figure 4E & F) despite the initial transient herbicide-induced decline in levels of this carotenoid.

As was expected, a water stress-induced rise in leaf ABA content occurred in both *A. byzantina* and *A. fatua* (Figure 5A & B). However, the rate of ABA accumulation in leaves of *A. fatua* was slower. Herbicide treatment caused a slight increase in ABA con-

tent of water-stressed leaves. Thereafter, ABA content of the leaves declined. A gradual loss of ABA was observed in leaves of *A. byzantina*, whereas in *A. fatua*, ABA declined rapidly to below basal levels. By comparasion, non-stressed leaves accumulated ABA throughout the course of the incubation period following herbicide treatment.

Discussion

The major symptomatic response following application of diclofop-methyl to leaves of susceptible species is inhibition of growth and onset of chlorosis. A reduction in photosynthetic CO_2-fixation, which is apparent after foliar application of diclofop-methyl, is therefore a secondary effect due to damage of the chloroplast membranes and associated reduction in chlorophyll content (Brezeanu *et al.* 1976). Similarly, alterations in carotenoid composition and ABA content also represent secondary events. Nevertheless, senescence of whole plants or leaves, which is a natural process, may also be considered the likely consequence of severe abiotic/biotic pressure, the most obvious characteristic being yellowing of leaves during the later stages of degradation. By inference, therefore, the consequence of herbicide application may be considered in terms of stress-induced senescence.

Results from the present study show that the diclofop-methyl-induced decline in photosynthetic CO_2 assimilation occurred concomitant with a decline in β-carotene and zeaxanthin content (Figures 2 & 4). Furthermore, the decline in carotenoid content coincided with a measurable increase in levels of ABA (Figures 3 & 5). It has recently been demonstrated for senescing leaves of *Hordeum vulgare* that loss of carotenoids is stoichiometrically related to an increase in ABA (Afitlhile *et al.* 1993). It is therefore not unreasonable to assume a similar metabolic interrelationship between carotenoid metabolism and ABA production in leaves of *Avena* species.

Perhaps the most significant observation to emerge from the current investigation was that in response to diclofop-methyl, zeaxanthin content of leaves of both species increased, following an initial decline (Figures 2E & F and 4E & F). This effect was, however, more dramatic in leaves of water-stressed plants. Accumulation of zeaxanthin following recovery of photosynthesis was associated with a reduction in leaf ABA content (Figures 2E & F, 3A & B, 4E & F and 5A & B). This phenomenon was less marked in leaves of *A. byzantina* and probably reflects the greater capacity of this species to metabolize ABA (Table 1). Although no measure of the turnover rate of ABA catabolism was made in the present study, little or no change in the level of ABA catabolites was detected in leaves of either *A. byzantina* or *A. fatua* following diclofop-methyl application (data not shown). ABA deficiency was not a consequence of diclofop-methyl in water-stressed *A. byzantina*, since basal levels were apparently re-established following the initial rise in leaf ABA content (Figure 3A). By comparison, leaf ABA content of *A. fatua* declined to below basal levels following application of the herbicide to water-stressed plants (Figures 3B & 5B). Such a reduction in leaf ABA content would be expected to impact on stomatal function and hence assimilation of CO_2.

There exists a close linear correlation between stomatal conductance and leaf ABA content (Steuer *et al.* 1988). Furthermore, reduced levels of ABA have been shown to greatly enhance both transpiration and photosynthetic CO_2 assimilation in leaves of *Vitis* (Loveys 1991). The present study revealed only slight differences in the gas exchange characteristics between the two species of wild oat examined. Although both had low light compensation points, typical of weeds, light saturation was observed at lower irradiance in *A. fatua*, which also displayed a lower maximum rate of photosynthesis. It was, therefore, unexpected that leaves of *A. fatua* should contain less ABA than *A. byzantina* when cultivated under control conditions (Table 1). Even so, the reduction in leaf ABA content of both species occurred concomitant with diclofop-methyl-induced accumulation of zeaxanthin and sustained or enhanced assimilation rates (Figure 2A & B). That net CO_2 assimilation rates were similar following the initial herbicide-induced decline for both stressed and non-stressed plants, was surprising. One possible explanation is that the diclofop-methyl- and/or water stress-induced increase in β,β-carotenoid synthesis may have contributed to increased photochemical efficiency by elevating the zeaxanthin content of the xanthophyll cycle in leaves of these plants. The above observations illustrate the complexity of secondary responses in leaves exposed to combined water stress and diclofop-methyl treatment and suggest that alterations in isoprenoid metabolism could contribute to increased fitness and hence reduced herbicide susceptibility in wild oat.

Seasonal use of AOPP graminicides does apparently cause wild-oat populations to evolve herbicide resistance (Heap *et al.* 1993). Although extensive studies have been carried out relating to the mechanism of tolerance/resistance to diclofop-methyl in *A. fatua* and several biotypes of this species (for review see Holt *et al.* 1993), it is perhaps more important to question the origin of the development of resistance to AOPP graminicides in wild oat, particularly as these species lack the insensitive procaryotic form of ACCase (Konishi & Sasaki 1994).

When soil starts to dry, one of the first responses shown by plants is a reduction in shoot growth. It is now apparent that an increase in ABA content leads to inhibition of shoot growth through its influence on stomatal conductance and leaf net CO_2 assimilation rates (Zeevaart & Creelman 1988). Where shoot growth is limited, demand for membrane acyl lipid synthesis through activity of ACCase is likewise reduced. Efficacy of ACCase-inhibiting graminicides like diclofop-methyl would therefore also be influenced. A herbicide/stress-induced reduction in ACCase activity might be expected to facilitate increased flux of carbon into isoprenoids. For example, zeaxanthin content is known to increase in leaves of water-stressed plants (Demmig-Adams & Adams 1992). Dehydration stress is also responsible for altering the complement of sterols and sterylglucosides in the plant plasma membrane. Since both sterols and carotenoids participate in regulating the thermodynamic and mechanical properties of membranes (Rohmer *et al.* 1979; Subczynski *et al.* 1993), an increase in capacity for terpenoid synthesis could form part of the mechanism responsible for the antagonistic effects of water stress on diclofop-methyl activity. There are good indications that ABA participates in the regulation of isoprenoid biosynthesis by feed-back inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity (Moore & Oishi 1994), the primary enzyme involved in the synthesis of terpenoids. A reduction in leaf ABA content due to stress-induced zeaxanthin accumulation would therefore be expected to contribute to increased synthesis of growth-promoting terpenoids such as gibberellins and brassinosteroids. Brassinosteroids, like gibberellins, stimulate internode elongation and show cytokinin-like activity (Mandava 1988). This suggests that changes in levels of these compounds may override the effect of AOPP herbicides, particularly in the presence of reduced ABA which is inhibitory to the mode of action of this class of compound.

Acknowledgements

This research was supported by a grant to one of us (A.K.C.) from Hoechst South Africa (Pty) Ltd. We also acknowledge

financial support from the Foundation for Research Development and Rhodes University. Mr Bill Goodwin is thanked for the supply of seeds of *Avena* species.

References

AFITLHILE, M.M., DENT, R.M. & COWAN, A.K. 1993. Changes in carotenoid composition in senescing leaves of *Hordeum vulgare* L. cv. Dyan. *J. Pl. Physiol.* 142: 43–49.

AKEY, W.C. & MORRISON, I.N. 1983. Effects of moisture stress on wild oat (*Avena fatua*) response to diclofop. *Weed Sci.* 31: 247–253.

ALBAN, C., BALDET, P., AXIOTIS, S. & DOUCE, R. 1993. Purification and characterisation of 3-methyl-crotonyl-coenzyme. A carboxylase from higher plant mitochondria. *Pl. Physiol.* 102: 957–965.

ANDREWS, M., DICKSON, R.L., FOREMAN, M.H., DASTGHEIB, F. & FIELD, R.J. 1989. The effects of different external nitrate concentrations on growth of *Avena sativa* cv. Amuri treated with diclofop-methyl. *Ann. Appl. Biol.* 114: 339–348.

BENNETT, J.M. 1990. Problems associated with measuring plant water status. *Hort. Sci.* 25: 1551–1554.

BREZEANU, A.G., DAVIS, D.G. & SHIMABUKURO, R.H. 1976. Ultrastructural effects and translocation of methyl-2-[4-(2,4-dichlorophenoxy)-phenoxy] propanoate in wheat (*Triticum aestivum*) and wild oat (*Avena fatua*). *Can. J. Bot.* 54: 2038–2048.

CAUSTON, D.R. & DALE, M.P. 1990. The monomolecular and rectangular hyperbola as empirical models of the response of photosynthetic rate to photon flux density, with applications to three *Veronica* species. *Ann. Bot.* 65: 389–394.

CHEN, Y., WURTELE, E.S., WANG, X. NIKOLAU, B.J. 1993. Purification and characterization of 3-methylcrotonyl-CoA carboxylase from somatic embryos of *Daucus carota*. *Arch. Biochem. Biophys.* 305: 103–109.

COBB, A. 1992. Herbicides in plant physiology. Chapman & Hall, London.

COWAN, A.K. & RAILTON, I.D. 1987. The catabolism of (±)abscisic acid by excised leaves of *Hordeum vulgare* L. cv. Dyan and its modification by chemical and environmental factors. *Pl. Physiol.* 84: 157–163.

COWAN, A.K. & RICHARDSON, G.R. 1993. The biosynthesis of abscisic acid from all-*trans*-β-carotene in a cell-free system from *Citrus sinensis* exocarp. *Pl. Cell Physiol.* 34: 969–972.

DASTGHEIB, F., ANDREWS, M., FIELD, R.J. & FOREMAN, M.H. 1990. Effect of different levels of manitol-induced water stress on the tolerance of cultivated oat (*Avena sativa* L.) to diclofop-methyl. *Weed Res.* 30: 171–179.

DEMMIG-ADAMS, B. & ADAMS III, W.W. 1992. Photoprotection and other responses of plants to high light. *Annu. Rev. Pl. Physiol. Pl. Mol. Biol.* 43: 599– 626.

DIEZ, T.A., WURTELE, E.S. & NIKOLAU, B.J. 1994. Purification and characterization of 3-methylcrotonyl-coenzyme-A carboxylase from leaves of *Zea mays*. *Arch. Biochem. Biophys.* 310: 64–75.

DUCKHAM, S.C., LINFORTH, R.S.T. & TAYLOR, I.B. 1991. Abscisic-acid-deficient mutants at the *aba* gene locus of *Arabidopsis thaliana* are impaired in the epoxidation of zeaxanthin. *Pl. Cell Environ.* 14: 601–606.

FIELD, R.J. & CASELEY, J.C. 1987. Abscisic acid as a protectant of *Avena fatua* L. against diclofop-methyl activity. *Weed Res.* 27: 237–244.

FOCKE, M., FELD, A. & LICHTENTHALER, H.K. 1991. Inhibition of early steps of de novo fatty acid biosynthesis by different xenobiotica. *Physiologia Pl.* 81: 251 – 255.

HEAP, I.M., MURRAY, B.G., LOEPPKY, H.A. & MORRISON, I.N. 1993. Resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in wild oat (*Avena fatua*). *Weed Sci.* 41: 232–238.

HOLT, J.S., POWELS, S.B. & HOLTON, J.A.M. 1993. Mechanisms and agronomic aspects of herbicide resistance. *Annu. Rev. Pl. Physiol. Pl. Mol. Biol.* 44: 203–229.

KOBEK, M., FOCKE, M. & LICHTENTHALER, H.K. 1988. Fatty-acid biosynthesis and acetyl-CoA carboxylase as a target of diclofop, fenoxaprop and other aryloxy-phenoxy-propionic acid herbicides. *Z. Naturforsch.* 43c: 47–54.

KONISHI, T. & SASAKI, Y. 1994. Compartmentation of two forms of acetyl-CoA carboxylase in plants and the origin of their tolerance toward herbicides. *Proc. Natl. Acad. Sci. USA* 91: 3598–3601.

LICHTENTHALER, H.K. 1990. Mode of action of herbicides affecting acetyl-CoA carboxylase and fatty acid biosynthesis. *Z. Naturforsch.* 45c: 521–528.

LOVEYS, B.R. 1991. How useful is knowledge of ABA physiology for crop improvement? In: Abscisic acid physiology and biochemistry, eds. W.J. Davies & H.G. Jones. pp. 245–260. Bios Scientific Publishers, Oxford.

MANDAVA, N.B. 1988. Plant growth-promoting brassinosteroids. *Annu. Rev. Pl. Physiol. Pl. Mol. Biol.* 39: 23–52.

MOORE, K.B. & OISHI, K.K., 3-Hyroxy-3-methylglutaryl coenzyme A reductase activity in the endosperm of maize *vivipary* mutants. *Pl. Physiol.* 105: 119– 125.

MOTEL, A., GÜNTHER, S., CLAUSS, M., FOCKE, M. & LICHTENTHALER, H.K. 1993. Studies on the inhibition of biotin-containing carboxylases by acetyl-CoA carboxylase inhibitors. *Z. Naturforsch.* 48c: 294–300.

OURISSON, G. 1994. Peculiarities of sterol biosynthesis in plants. *J. Pl. Physiol.* 143: 434–439.

POPJAK, G. 1971. Specificity of enzymes of sterol biosynthesis. *Harvey Lect.* 65: 127–165.

QUARRIE, S.A. 1991. Implications of genetic differences in ABA accumulation for crop production. In: Abscisic acid physiology and biochemistry, eds. W.J. Davies & H.G. Jones, pp. 227–243. Bios Scientific Publishers, Oxford.

ROCK, C.D. & ZEEVAART, J.A.D. 1991. The *aba* mutant of *Arabidopsis thaliana* is impaired in epoxy-carotenoid biosynthesis. *Proc. Natl. Acad. Sci. USA* 88: 7496–7499.

ROHMER, M., BOUVIER, P. & OURISSON, G. 1979. Molecular evolution of biomembranes. Structural equivalents and phylogenetic precursors of sterols. *Proc. Natl. Acad. Sci. USA* 76: 847–851.

ROSSI, F.S., DI TOMASO, J.M. & NEAL, J.C. 1993. Fate of fenoxaprop-ethyl applied to moisture-stressed smooth crabgrass (*Digitaria ischaemum*). *Weed Sci.* 41: 335–340.

STEUER, B., STUHLFAUTH, T. & FOCK, H.P. 1988. The efficiency of water use in water stressed plants is increased due to ABA induced stomatal closure. *Photosyn. Res.* 18: 327–336.

SUBCZYNSKI, W.K., MARKOWSKA, E. & SIELEWIESIUK, J. 1993. Spin-label studies on phosphatidylcholine-polar carotenoid membranes: effects of alkyl-chain length and unsaturation. *Biochim. Biophys. Acta* 1150: 173–181.

ZEEVAART, J.A.D. & CREELMAN, R.C. 1988. Metabolism and physiology of abscisic acid. *Annu. Rev. Pl. Physiol. Pl. Mol.* 439–473.